IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants :	Dotan et al.		
Serial Number :	10/728,227	Examiner :	James Leslie Grun
Filing Date :	December 3, 2003	Art Unit :	1641
For:	Method for Diagnosing Diseases Based on Levels of Anti-Glycan Antibodies		

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Declaration of Nir Dotan under 37 C.F.R. § 1.132

I. Nir Dotan, Ph.D., declare and state:

1. I am a joint inventor of the subject matter described and claimed in United States Patent Application Serial No. 10/728,227, filed December 3, 2003 ("the '227 application"), entitled "Method for Diagnosing Diseases Based on Levels of Anti-Glycan Antibodies".

2. I am presently employed as the Chief Technology Officer and Vice President, Research & Development, of Glycominds, Ltd., the assignee of the above-referenced application.

3. I received my Ph.D. from Tel-Aviv University, Tel Aviv, Israel, in the field of protein-carbohydrate interactions. I have 15 issued patents and 10 articles published in peer reviewed journals in the field of glycan-protein interactions and glycan biomarkers for clinical diagnosis and prognosis.

4. I have re-read the specification and claims of the above-referenced patent application, the January 5, 2007 Office Action, and the July 11, 2007 Office Action, and the references cited in these Office Actions. I understand that the Examiner has rejected the claims as anticipated by

Main *et al.* BMJ 297:1105, 1988 ("Main") in light of our disclosure and/or Sendid *et al.*, Clin. Diagn. Lab. Immunol. 3:219, 1996 ("Sendid"), and/or Wakshull *et al.*, US Patent No. 6,294,321 ("Wakshull"), and as anticipated by Sendid in light of our disclosures and Wakshull. I do not agree with the Examiner's rejection of the claims, and I provide this Declaration to explain why the claims are novel and non-obvious over the cited art.

5. The claimed invention provides a sensitive serological testing method for definitively distinguishing Crohn's Disease (CD) from other digestive diseases. The highly sensitive primary screening assays according to the invention provide physicians with an inexpensive assay for rapidly distinguishing individuals with CD from non diseased individuals, or individuals having disease with symptoms similar to CD, such as ulcerative colitis (UC) or inflammatory bowel syndrome (IBS). This facilitates earlier and more appropriate therapeutic intervention and minimizes uncertainty for patients and their families.

6. The invention is based in part on the discovery that patients with CD have higher serum levels of certain IgG and IgA isotype antibodies specific for certain defined glycan structures as compared to the serum levels of these antibodies in healthy individuals or in individuals with other types of gastrointestinal diseases.

7. Prior to our invention it had been recognized that antibodies to mannan polysaccharide from certain *Saccharomyces cerevisiae* species are present in the sera of CD patients, (Sendid et al., Clin Diagn Lab Immunol. 1996 Mar;3(2):219-26)). However, neither Sendid nor any other researcher has shown that any antibodies to other oligomannosides, other polysaccharide, or any other defined glycan fragments (oligosaccharides), are present in CD patients.

8. In fact, few additional non-glycan (protein based) antigens have subsequently been associated with CD. Since 1999 several companies have developed commercial anti-*Saccharomyces cerevisiae* antibodies (ASCA) tests, such as Prometheus Laboratories Inc. (San Diego, CA) and Inova Diagnostics Inc. (San Diego, CA). Even though those companies were successful in the commercialization of their ASCA test and also devoted time and money to identifying additional novel microbial antigens, only protein based microbial antigens were found, such as I-2 from *Pseudomonas fluorescens*, Outer membrane of E-coli (OmpC and Omp+

respectively), and anti-CBir1,which is an antibody against flagellin expressed by a certain *Clostridial phylum* species. None of these companies identified any additional glycan-based antigens.

9. The instant application differs from this and other prior art in that it is based on a method that diagnoses CD based on antibodies to defined glycan structures, as opposed to antibodies to polysaccharide and one of its oligosaccharide components of a yeast cell (i.e. mannan and oligomannoside, respectively). The instant application was filed based on discoveries from screening a library of defined glycans (complex carbohydrates) attached onto a solid phase (an array) according to methods described in co-owned US Patent No., 6,972,172 with sera samples from CD and control patients. We show in the instant application that the defined glycan structures recited in the claims are specifically recognized by antibodies in sera of patients with CD. These results demonstrate that CD patients react differently to defined glycans as compared to their reactivity to ASCA, which is based on a mannan antigen . In only 25% of the samples tested were both antibodies to defined glycans (i.e. anti-laminaribioside antibodies), and ASCA detected. Only 11% of the samples tested had both anti-chitobioside antibodies and ASCA present.

10. The Examiner has rejected the claims as anticipated by Main in light of Sendid, our own disclosure, or Wakshull, and as anticipated by Sendid et al. in light of Wakshull. I understand that the Examiner contends Main teaches IgG and IgA antibodies to a crude extract (ASCA) are detected in the circulation of patients with CD, but not in patients with ulcerative colitis (UC). I understand that the Examiner alleges Sendid teaches that antibodies to phosphopeptidomannans from certain *Saccharomyces cerevisiae* species are detected in the circulation of CD patients, but not in patients with UC. I understand that the Examiner asserts that assays as described in these references inherently detect antibodies to glycan epitopes, such as oligomannoside and mannans, present in yeast cells. I also understand that the Examiner alleges Wakshull teaches the detection of soluble β (1-3)-glucans in the blood of patients suffering from fungal infections by immobilized anti β (1-3)-glucans monoclonal antibody, and that this antibody have cross reactivity with laminaribioside disaccharide. I disagree with this conclusion, for at least three reasons.

11. First, the Examiner asserts that Main and Sendid teach that antibodies to *Saccharomyces cerevisia* (ASCA) species are specific to sera from Crohn's Disease (CD) patients. However, Sendid found that not all *Saccharomyces cerevisiae* strains tested reacted with sera from CD patients. Although phosphopeptidomannan exists in the cell wall of all *Saccharomyces cerevisiae*, only *Saccharomyces cerevisiae* strain Su1 and *Saccharomyces cerevisia* Sd are reactive to CD sera. Other strains tested by Sendid--*Saccharomyces cerevisiae* BM156, *Saccharomyces cerevisiae* BM151, and *Saccharomyces cerevisiae* CBS1315 are not specific antigens to sera from CD patients. Thus, the data in Sendid do not support a conclusion that antibodies in CD patients always detect epitopes in *Saccharomyces cerevisiae* strains, which can differ in their outer membrane glycan antigens epitopes.

12. Second, Wakshull describes a method for analyzing the presence of soluble β (1-3)-glucans in blood as a form of debris from fungi, including yeast cell walls. Monoclonal antibodies used to detect this beta-glucan are attached to a solid phase and thus identify the glycan structure in the circulation system of patients with fungal infections. While Wakshull is apparently cited for describing β (1-3) glucans and mannans as components of yeast cell cells, it does not discuss CD, nor does it teach diagnosing CD using antibodies to defined glycans. In contrast, our claimed methods require detecting the level of natural antibodies in serum of CD patients by using a defined beta-glucan disaccharide as the probe. Wakshull is apparently cited as well for teaching that a monoclonal antibody that recognizes a beta –glucan can cross react with one of its sub fragments, Glc(beta1,3)Glc(beta) laminaribioside. However, it does not follow that if polyclonal antibodies to polysaccharide exists in sera of CD patients and are specific to CD, then antibodies to its defined sub fragment will exist and will be specific for the same clinical condition. This is true for at least three reasons:

12.1 Beta glucans are polysachhrides composed of a glucose monosaccharide connected in beta 1,3 or beta 1,6 glycosidic bonds, thus creating a complex and branched structure with un predictable three dimensional structure. One cannot predict in the absence of experimentation which sub fragment glycan epitopes will be exposed on the surface of the polysaccharide, how many of each sub fragment exists, or whether the glycan sub fragment will trigger an immune response to this specific epitope and at what level.

12.2. Further experiments performed by the Assignee of this application demonstrate the unpredictable nature and lack of correlation between antibody reactivity to polysaccharides and to defined fragments thereof. In the following experiments, which are disclosed in USSN 11/351,185, a continuation-in-part of the instant application, anti glycan antibodies levels to a set of two polysaccharides, and fragments thereof were measured in sera samples of CD patients and controls using ELISA. Patient population samples and antibodies tested are described in the following table:

Population tested	**Disaccharide fragment of the polysaccharide**	**Polysaccharide**	**Antibody isotype**
German cohort: CD (n=133) Control samples (75 UC)	Glc(b1,3)Glb(b) (ALCA)	Beta Glucan (laminarin)	IgG
Canadian cohort: CD (n=551) Control samples (334 UC, 193 healthy controls, 30 IBS, Total 545)	GlcNAc(b1,4)GlcNAc(b) (ACCA)	Chitin	IgA

The correlation between anti-polysaccharides and their fragment was calculated using Pearson methods and reported as R^2.

12.2.1. The following Figure 1 describe the results of the comparison. No correlation was observed between reactive to laminarin (beta-glucan based polysaccharide) and to the defined glycan Glc(b1,3)Glc(b) ($R^2 = 0.17$).

12.2.2. A similar lack of correlation and un predictable nature was further observed between antibodies reactive to chitin (aGlcNAc based polysaccharide) and the defined disaccharide fragment glycan GlcNAc(b1,4)GlcNAc(b) disaccharide ($R^2 = 0.15$), see Figure 2.

Figure 1.

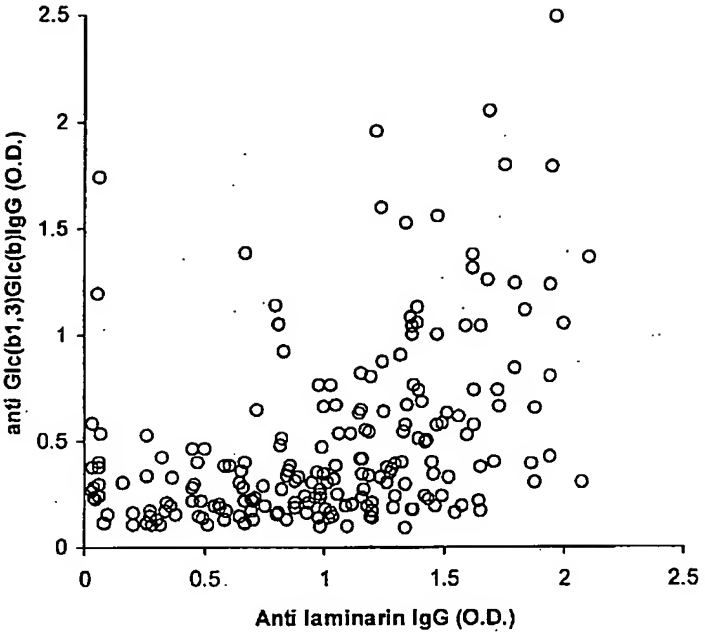
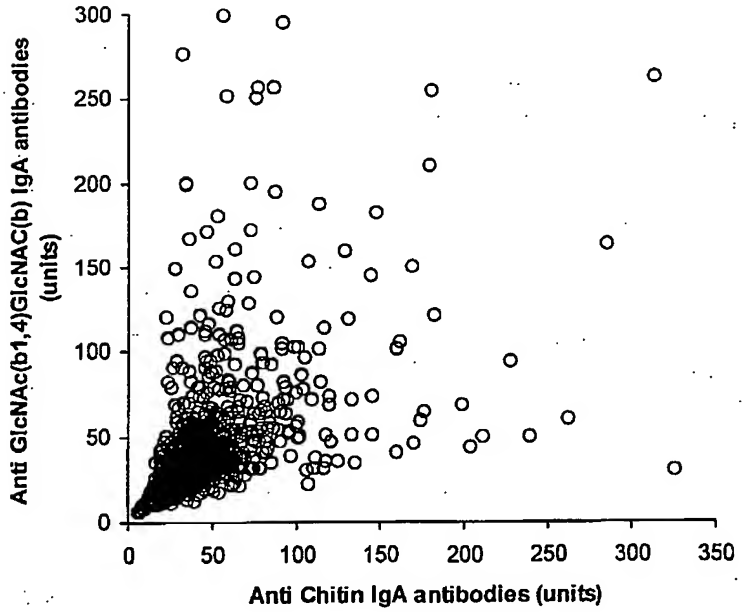


Figure 2.



12.2.3. These results demonstrate a lack of correlation (R^2 0.15- 0.17) between anti-glycan antibodies reactive to polysaccharides or fragments of the polysaccharides. In both cases there are patients who are highly reactive towards the polysaccharide but not reactive towards the defined sub fragment and vice versa, demonstrating the unpredictable nature of the association. Therefore, one cannot assume that if an individual has developed an immune response to a polysaccharide, he will also react immunologically to a sub fragment of the polysaccharide.

12.3. The lack of correlation between an immune response to *Saccharomyces cerevisiae* (ASCA) and an immune response to any other glycan sub fragment that may exist in *Saccharomyces cerevisiae* has been well demonstrated in several scientific publications. Dotan, I et al. (Dotan, I et al. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. Gastroenterology. 2006 Aug;131(2):366-78.) and Ferrante et al. (Ferrante et al. New serological markers in inflammatory bowel disease are associated with complicated disease behavior. Gut. 2007 Apr 24; [Epub ahead of print]), both studies based on large IBD populations, report that ASCA reactivity does not necessarily accompany immune reactivity towards other defined glycan structures such as ALCA, ACCA and AMCA. Figures 3 and 4 are the original Venn diagrams taken from Dotan I. et al. and Ferrante et al. respectively, and describe the existence of ALCA ACCA or AMCA positive patients in the population of CD patients who are ASCA negative. Therefore, one cannot assume that if reactivity to a polysaccharide from *Saccharomyces cerevisiae* exists, then reactivity to other glycan exists as well.

Figure 3. Venn Diagram; Relationship between ALCA, ACCA, and ASCA in the CD (n=72) cohort by presence versus absence. Figure 2 in Dotan I. et al., A total of 44% of ASCA-negative CD patients was positive for ALCA or ACCA.

GASTROENTEROLOGY 2006;131:368–378

Antibodies Against Laminaribioside and Chitobioside Are Novel Serologic Markers in Crohn's Disease

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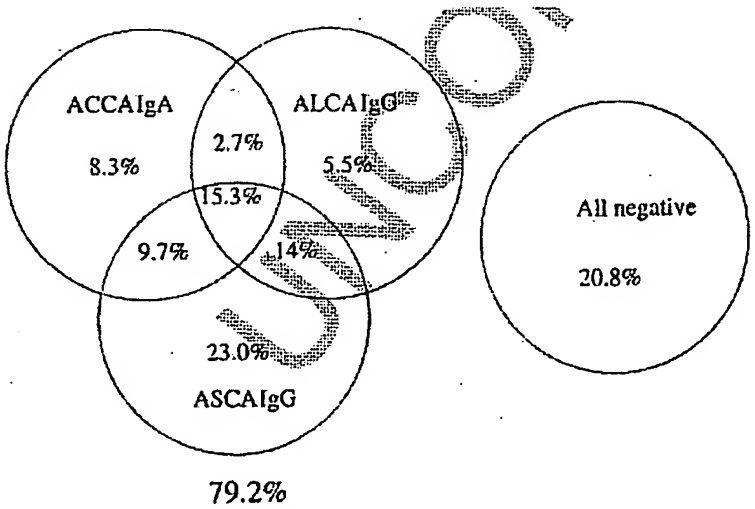

Figure 2. Relationship between ALCA, ACCA, and ASCA in the CD cohort by presence vs absence. Percentage of the CD patient cohort (n = 72) that is positive for each marker, any combination of 2 markers, and all markers are shown. ALCA and ACCA were determined by the glycan array and ASCA by the commercial ASCA kit (Inova Diagnostics), as described in the Materials and Methods section. A total of 44% of ASCA-negative CD patients was positive for ALCA or ACCA.

Figure 4 – Venn Diagram ; Presence of glycan-antibodies ALCA, ACCA and AMCA in a cohort of 398 ASCA negative CD patients. (Figure 2 in Ferrante et al.) . A total of 32.4% of the ASCA negative patients are positive for ALCA, ACCA or AMCA

GUT ONLINE

New serological markers in inflammatory bowel disease are associated with complicated disease behaviour

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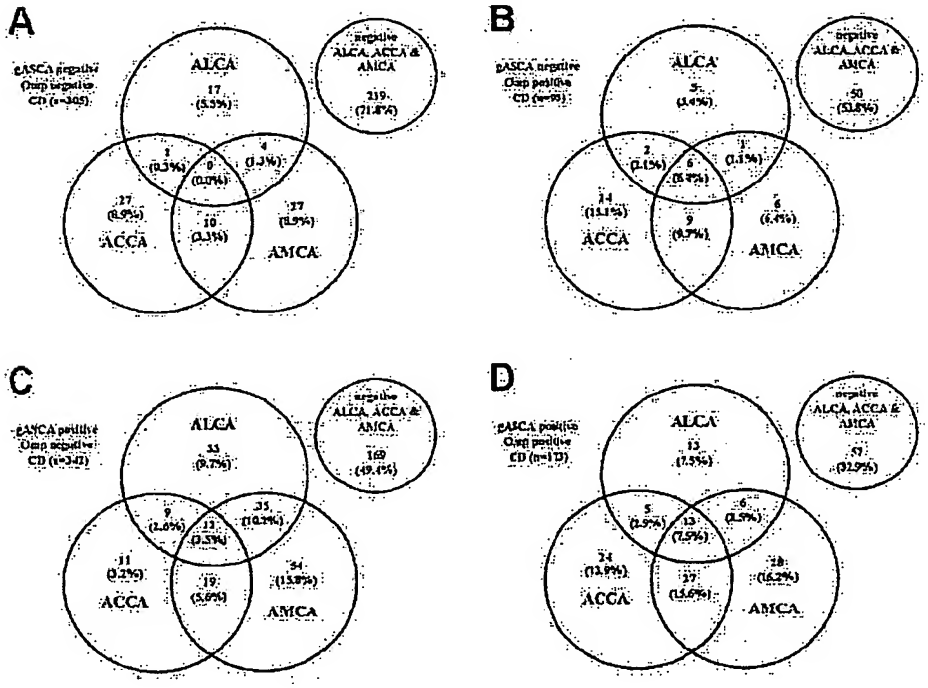


Figure 2. Presence of glycan-antibodies ALCA, ACCA and AMCA in 305 gASCA negative Omp negative CD patients (Fig 2A), 93 gASCA negative Omp positive CD patients (Fig 2B), 342 gASCA positive Omp negative CD patients (Fig 2C), and 173 gASCA positive Omp positive CD patients (Fig 2D).

13. More recently obtained data has shown than the antigens triggering elevated levels of ALCA are not related to *Saccharomyces cerevisiae*, but rather related to different *Candida albicans*. Experiments performed by Drs. D. Poulain, and B. Sendid form the Department of Microbiology in Lille University Hospital (France), show that ALCA develop in rabbits as result of injection of live *Candida albicans* cells that cause systemic infection, see Figure 5. Furthermore, high levels of ALCA are found in sera samples taken from human patients with systemic *Candida albicans* infection (Systemic Candidiasis, SC , n=69) in comparison to healthy controls (HC, n=32), see Figure 6. This data clearly demonstrate that antigens triggering elevated levels of ALCA in CD pateints are not related to *Saccharomyces cerevisiae* , but rather originated from exposure to *Candida albicans* cells (exists in natural human intestine flora) crossing the leaky bowel of CD patients. Therefore it is not obvious to assume that antibodies to *Saccharomyces cerevisiae* antigens, other then mannan, will be specific to CD patients.

Figure 5. Development of ALCA in three New Zealand white rabbits (2–3 kg) that were inoculated intravenously with 500 µL of suspensions of live cells of *Candida albicans* strain VW32, $2*10^6$ cells/mL.

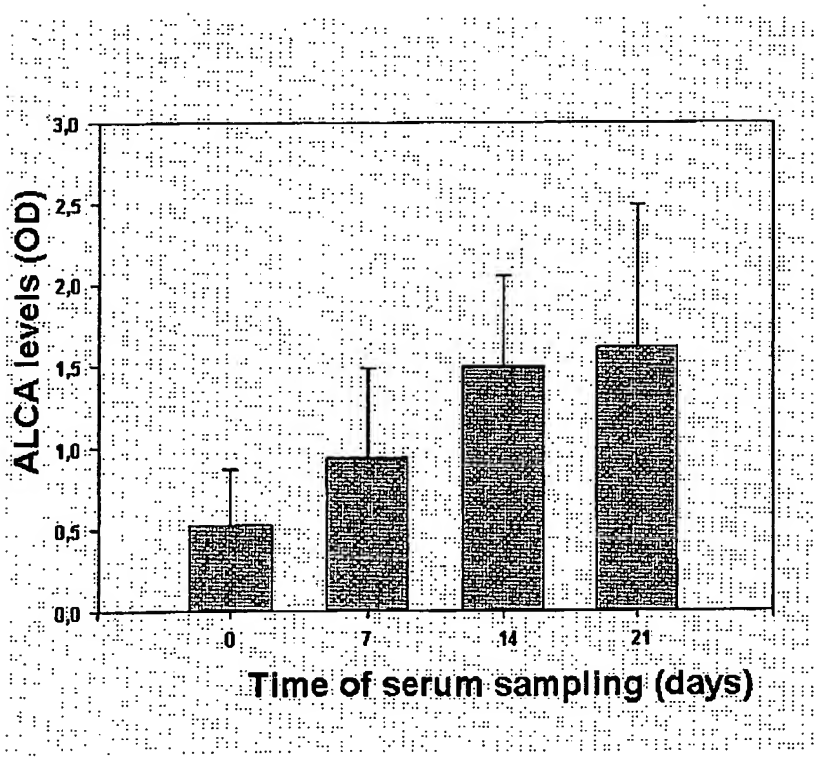
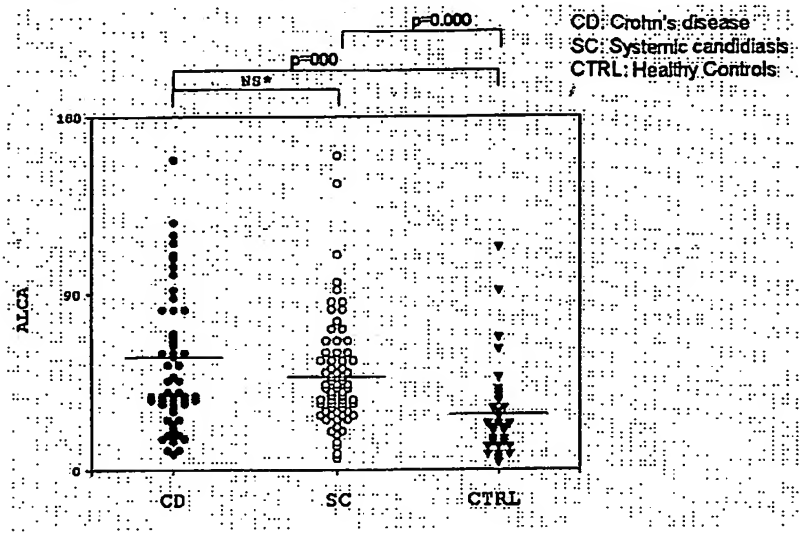


Figure 6. ALCA levels in SC in comparison to CD patients and healthy controls



14. The novelty and non- obviousness of the claimed invention has been recognized by others in the field. An editorial review appearing in the same issue of Gastroenterology journal as a post-filing to the Dotan I. et al. publication of our claimed invention stated:

> The first interesting aspect of this study is the methodology used to identify these antibodies. Glycans are composed of saccharide building blocks and have great combinatorial diversity based on the number of different linkages possible between saccharides. These investigators developed a glycan array consisting of mono- and oligosaccharides bound to a solid support by way of a long linker. This set-up permits the glycan to be presented to antibodies in an orientation found in native glycoconjugates, for example in bacterial cell walls. Using this glycan array, they first identified which glycan moieties were recognized by antibodies present in the serum of patients with inflammatory bowel disease. In addition to the 2 studies described previously, Crohn's disease patient sera also reacted with mannans such as those used in the ASCA assay and provided internal validity for this approach. They next developed enzyme-linked immunosorbent assay (ELISA) assays for laminaribioside and chitobioside to permit larger-scale analyses.... Importantly, ALCA or ACCA were positive in 44% of patients in whom ASCA was negative, so it could help detect additional patients not identified by ASCA as a stand alone test. ...

15. In addition, formation of ALCA, ACCA and gASCA was reported to be associated with different variants in innate immune receptor genes in CD pateints. (Henchaets et al., Gut 2007 June 2006; [Epub ahead of print] (copy enclosed). Positivity to gASCA was found to be associated with variants of CARD15 and CARD4 genes, where as positivity to ALCA was found to be associated only with CARD15 (see figure 1 A-D in Henchaets et al. , In figure 7 below). However, positivity to ACCA was found to be associated with variants of a completely different gene, TLR4 (see figure 2 A in Henchaets et al. , In figure 7 below). The variability in association between antibody reactivity (ASCA, ALCA, or ACCA) with different genotypes further illustrates the unpredictability of associating antibodies to glycan structures and CD or related diseases.

Figure 7.

GUT ONLINE

Mutations in pattern recognition receptor genes modulate seroreactivity to microbial antigens in patients with inflammatory bowel disease

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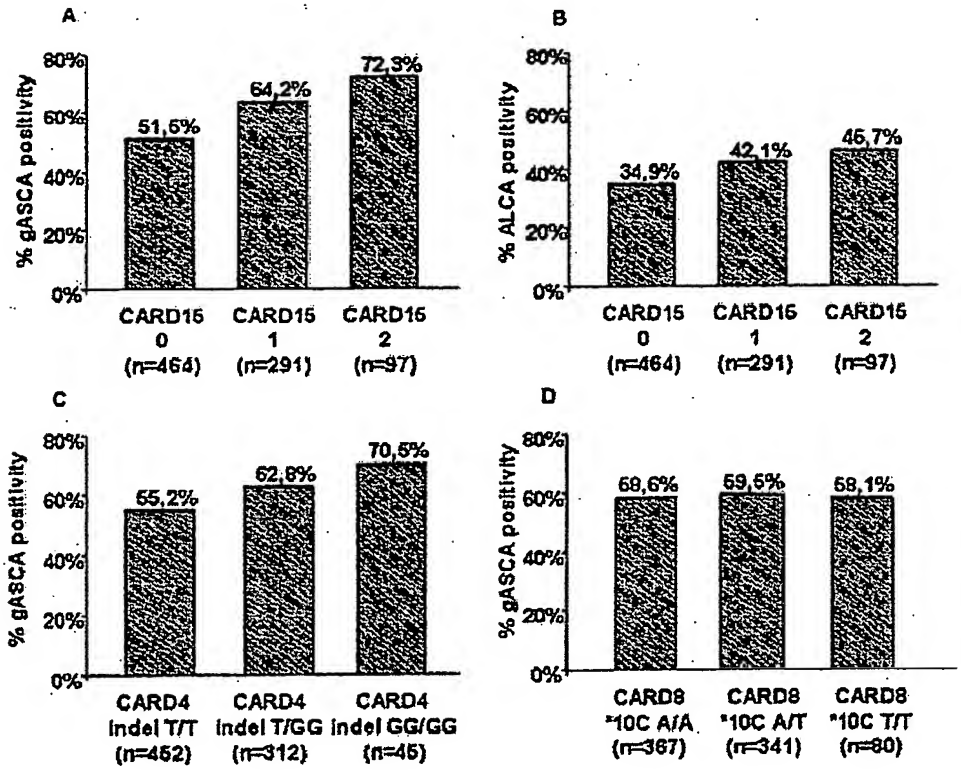

Figure 1 A-D. Antibody positivity in CD patients, according to their CARD15, CARD4 and CARD8 status. Panel A: gASCA and CARD15, overall p-value <0.0001. Panel B: ALCA and CARD15, overall p-value 0.04. Panel C: gASCA and CARD4 indel, overall p-value 0.03. Panel D: gASCA and CARD8, overall p-value 0.3.

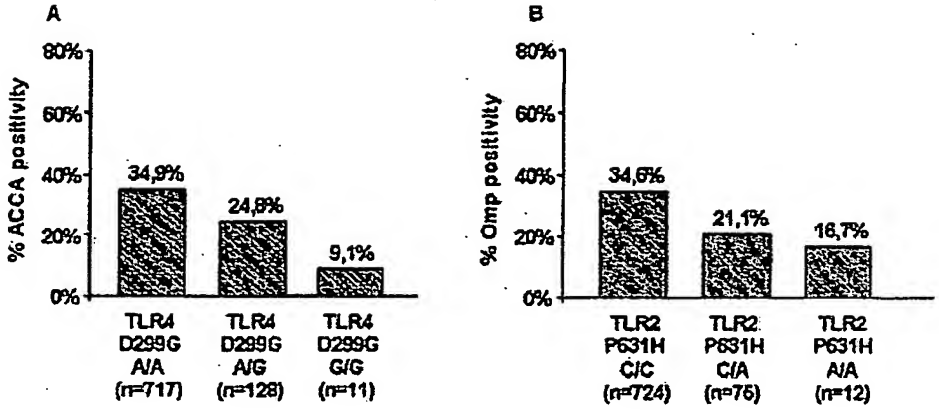

Figure 2 A-B. Antibody positivity in CD patients, according to their TLR4 and TLR2 status. Panel A: ACCA and TLR4 D299G, overall p-value <0.026). Panel B: Omp and TLR2 P631H, overall p-value 0.035).

16. I further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Nir Dotan, Ph.D.

10th January 2008
Date

Scientific publication in the field

Nir Dotan. In: Autoantibodies, Y Shonfeld,M. E. Gershwin, and P.L. Meroni (editors). Anti-glycan antibodies. Elsevier B.V. Burlington. 2007 (pp.817-822).

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Liesbet CM Henckaerts, Marie Pierik, Marie Joossens, Marc Ferrante, Paul J Rutgeerts and Severine Vermeire patients with inflammatory bowel disease modulate seroreactivity to microbial antigens in Mutations in pattern recognition receptor genes. Gut published online 26 Jun 2007

Granted patents in US and Europe in the field

- US6972172,
- US6994966
- EP1153298B1

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